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Representation of behaviourally relevant information by blowfly motion-sensitive visual interneurons requires precise compensatory head movements

R. Kern[1,*], J. H. van Hateren[2] and M. Egelhaaf[1]

[1]*Department of Neurobiology, Faculty for Biology, Bielefeld University, Bielefeld 33501, Germany and*
[2]*Department of Neurobiophysics, University of Groningen, Groningen 9747 AG, The Netherlands*

*Author for correspondence (e-mail: roland.kern@uni-bielefeld.de)

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Summary

Flying blowflies shift their gaze by saccadic turns of body and head, keeping their gaze basically fixed between saccades. For the head, this results in almost pure translational optic flow between saccades, enabling visual interneurons in the fly motion pathway to extract information about translation of the animal and thereby about the spatial layout of the environment. There are noticeable differences between head and body movements during flight. Head saccades are faster and shorter than body saccades, and the head orientation is more stable between saccades than the body orientation. Here, we analyse the functional importance of these differences by probing visual interneurons of the blowfly motion pathway with optic flow based on either head movements or body movements, as recorded accurately with a magnetic search coil technique. We find that the precise head–body coordination is essential for the visual system to separate the translational from the rotational optic flow. If the head were tightly coupled to the body, the resulting optic flow would not contain the behaviourally important information on translation. Since it is difficult to resolve head orientation in many experimental paradigms, even when employing state-of-the-art digital video techniques, we introduce a 'headifying algorithm', which transforms the time-dependent body orientation in free flight into an estimate of head orientation. We show that application of this algorithm leads to an estimated head orientation between saccades that is sufficiently stable to enable recovering information on translation. The algorithm may therefore be of practical use when head orientation is needed but cannot be measured.

Key words: optic flow, motion sensitive neuron, eye movement, blowfly, *Calliphora vicina*.

Introduction

Virtually all animals with developed visual systems are able to actively control their gaze using eye, head and/or body movements (Land, 1999; Land and Collett, 1997; Findlay and Gilchrist, 2003). Active vision ensures that relevant visual information is available when it is needed and may simplify a variety of otherwise difficult computational tasks. One such task is to acquire detailed visual information from objects of particular interest, which requires stabilisation of the retinal image for some time. The majority of animals accomplish this task by a pattern of stable fixations with interspersed fast saccades that shift the gaze direction to objects of interest. During fixation periods, a relatively unblurred image is provided, at least in a small region of the visual field (Land, 1999; Land and Collett, 1997; Findlay and Gilchrist, 2003). However, detailed object vision is not the only reason why a saccadic gaze strategy is useful for visual information processing. Animals can acquire spatial information on the surroundings by analyzing optic flow – the pattern of motion generated on the eyes while an animal moves around in its environment. Gaze control by eye, head and/or body movements is also important for optic flow processing: here, saccadic gaze shifts are a means to concentrate the optic flow components caused by rotation into short periods, the saccades. As a consequence, the periods between saccades consist primarily of translational optic flow. Such a translational optic flow field is most useful, because local motion velocities in this field depend on the distance of the animal to environmental objects. Rotation, by contrast, causes all motion vectors to have a direction and size independent of object distances. Hence, for judging distances to objects, the most suitable optic flow is optic flow purely caused by translation, i.e. free from contamination by rotational image motion (Gibson, 1979; Lappe, 2000; Vaina et al., 2004; Land, 1999).

Blowflies employ a saccadic gaze strategy and show saccadic movements of both body and head (Land, 1973; Wagner, 1986a; Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999; R. Kern, unpublished results). Since in blowflies the eyes are fixed to the head, the orientation of the head in space reflects the direction of gaze. During free flight,

the yaw angles of head and body, i.e. the rotation angles around a vertical axis, roughly coincide during saccades. Nonetheless, there are differences in detail: the head starts turning slightly later, but reaches its final orientation somewhat earlier, than the body (van Hateren and Schilstra, 1999). As a consequence, the head intersaccadic interval is longer than that of the body. This extension of the time interval available for the evaluation of translational optic flow is rendered possible because of very precise head–body coordination. Since saccades are generated during spontaneous flight at rates of up to 10 per second, this head–body coordination has to work on a very short timescale, i.e. within milliseconds.

Here, we address the functional significance of the subtle differences between body and head movements for the evaluation of optic flow by the fly motion pathway. This question can be addressed in blowflies by using a sophisticated system to monitor body and head movements in free flight (Schilstra and van Hateren, 1998). From such measurements it is possible to determine in great detail what (1) flies have actually seen during a flight and (2) what they might have seen if the yaw orientation of their head had been constantly aligned with their body long axis. Moreover, in the blowfly visual motion pathway, individually identified interneurons, which are involved in optic flow processing and in controlling visually guided orientation behaviour, are well amenable to electrophysiological analysis (Hausen and Egelhaaf, 1989; Krapp, 2000; Egelhaaf et al., 2002; Egelhaaf and Kern, 2002; Borst and Haag, 2002; Egelhaaf et al., 2005). Utilizing novel visual stimulation techniques (Lindemann et al., 2003), we could show previously that several of these motion-sensitive neurons represent, between saccades, information about translational optic flow and thus, indirectly, about the animal's self-motion and the spatial layout of the environment (Kern et al., 2005; van Hateren et al., 2005). Here, we will show that this information is no longer readily available in the neuronal responses, if it is assumed that the yaw angle of the head is identical to that of the body. Hence, the precise head and body coordination between saccades is highly relevant from a functional point of view.

Most behavioural studies employ video techniques and in most paradigms the video data do not allow the experimenter to accurately resolve head orientation. Accurate head orientation would be required if the optic flow received by the observed animal is to be determined. As a practical solution to this problem, we present here an algorithm that recovers, to some extent, the most relevant features of head movements from measurements of body movements. This algorithm is likely to be useful, if – for technical reasons – optic flow has to be determined based only on body orientation.

Materials and methods

Stimulus generation and electrophysiology

All experiments were done on the blowfly *Calliphora vicina* (Robineau-Desvoidy). All visual stimuli were derived from behavioural data based on blowflies flying in a cage of about $40\times40\times40$ cm^3, with images of herbage on its side walls. The position and orientation of the head and of the body (thorax) of the blowflies were recorded using magnetic fields driving search coils attached to the flies. Since this method has already been described in detail in previous studies (Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999), it will be only briefly summarised here. Small sensor coils made of copper wire are mounted on the thorax and on the head of the fly. Large field coils surrounding the flight cage produce time-varying magnetic fields, which induce voltages in the sensor coils. These voltages are transferred *via* a bundle of very thin wires (wire diameter, 12 μm) to amplifiers. The wire bundle runs loosely from the abdomen of the fly to the bottom of the cage and is carried around by the flying insect. In a series of control experiments (Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999) it was established that the wire bundle and the coils do not interfere noticeably with normal flight behaviour and normal head movements.

Three approximations to natural, behaviourally generated optic flow were used in the electrophysiological experiments. They are based on different types of behavioural data. (1) *Head movements*. The position and orientation of the head is used for determining the image sequences. Because the fly's compound eye is an integral part of its head, and the visual interior of the cage is known, the visual stimulus encountered by the fly during a flight could be reconstructed. (2) *Body yaw*. For determining the retinal image sequences, only the position is used from the head data. The yaw orientation is taken from the body long axis, the head pitch angle is assumed to be fixed at the average value we measured for the head pitch in the particular flight (20.3° and 25.8° upwards from the horizontal plane for the two flights used here), and the head roll angle is fixed at 0°. In other words, the head is assumed to be aligned with the body and perfectly stabilized against roll and pitch movements of the body. When behavioural data are obtained with cameras, such an approximation is usually made because of resolution limits. (3) *Tuned body yaw ('headified')*. Although the parameters of head and body yaw movements are similar, they differ in many important respects (van Hateren and Schilstra, 1999). Since these will be shown to have significant consequences for the neuronal responses, a filtering procedure was developed to tune the measured body movements, i.e. to make the filtered data a good approximation of the real head movements. Since it is one result of the present study that such a filtering procedure leads to an acceptable approximation of head movements, the procedure will be described in the Results section. The source code of the algorithm will be made available by the authors on request.

Two flights, each of 3.45 s duration, originating from two different flies, were used for stimulus reconstruction. In the electrophysiological experiments, the image sequences were replayed on a panoramic stimulus device (FliMax; Lindemann et al., 2003) at a frame rate of 370 Hz. Proper spatial and temporal prefiltering prevented spatiotemporal aliasing during fast turns. An approximation of the response

of the corresponding visual interneurons in both brain hemispheres to the same flight was obtained by presenting a mirrored version of the reconstruction. Intracellular recordings were made from the horizontal system neurons HSN (north), HSE (equatorial) and HSS (south) (Hausen, 1982a; Hausen, 1982b) and the dorsal centrifugal horizontal neuron (DCH) (Hausen, 1976; Haag and Borst, 2002) in the right optic lobe of 1–2-day-old female blowflies following standard routines (Warzecha et al., 1993), with careful alignment of the flies' eyes. Results are based on HSE recordings from four flies, on HSS recordings from three flies, on HSN recordings from two flies and DCH recordings from three flies. Experiments were done at temperatures between 28 and 32°C, measured close to the position of the fly in the centre of FliMax. These temperatures are in the range of head temperatures measured in flying blowflies (Stavenga et al., 1993).

Data analysis

The data analysis followed closely the procedure described before (Kern et al., 2005; van Hateren et al., 2005). Briefly, coherence between a self-motion parameter of the blowfly, i.e. either yaw velocity or sideward velocity, and the responses was calculated as $\gamma_b^2=|P_{sr}|^2/(P_{ss}P_{rr})$ (Theunissen et al., 1996), where P_{sr} is the cross-spectral density of the motion parameter and response, P_{ss} is the power spectral density of the motion parameter, and P_{rr} is the power spectral density of the response. Spectra were calculated by periodogram averaging of 50% overlapping data segments, with each periodogram being the discrete Fourier transform of a $\cos^2$-tapered zero-mean data segment of 256 ms, extended by zero-padding to 512 ms. Results were not strongly dependent on segment length. Before segmentation, the response was aligned with the self-motion parameter by shifting it 22.5 ms backwards in time, the approximate latency under the experimental conditions. Results were not strongly dependent on shift size. Segments from all flights used as stimulus for a particular cell were included in the periodogram averaging. Coherence of the response with two self-motion parameters was obtained by first conditioning the second parameter with the first (Bendat and Piersol, 2000), i.e. $s_2'=s_2-(P_{21}/P_{11})s_1$, where s_1 is the first parameter, and s_2 and s_2' are the original and conditioned second parameter, respectively; P_{21} and P_{11} are cross and power spectra of the second and first parameter. Conditioning removes the second-order statistical dependence with s_1 from s_2. We found that the order of evaluating parameters does not significantly affect the results for the stimulus parameters used in this study.

Since the responses in the intersaccadic intervals were previously found to be particularly interesting with respect to representing specifically translational self-motion parameters (Kern et al., 2005), coherences between the self-motion parameters and the corresponding responses in the intersaccadic intervals were obtained by masking the regions surrounding saccades. For further details, see (van Hateren et al., 2005; Kern et al., 2005).

Results

Differences in gaze and body orientation

During spontaneous flights, flies do not turn smoothly. They rather perform rapid changes in the orientation of their body long axis. Between these saccades, the orientation is relatively stable (Fig. 1A, red trace), and the turning velocity is consequently much lower. The differences in yaw velocity of saccades and intersaccadic intervals usually allow us to pinpoint saccades as distinct events. Head saccades go along with body saccades and are characterised by even faster changes in the yaw angle (Fig. 1A, blue trace) than the body saccades. Between saccades, the yaw angle of the head changes much less than that of the body (Fig. 1A). The body orientation often drifts during most of the intersaccadic interval into the

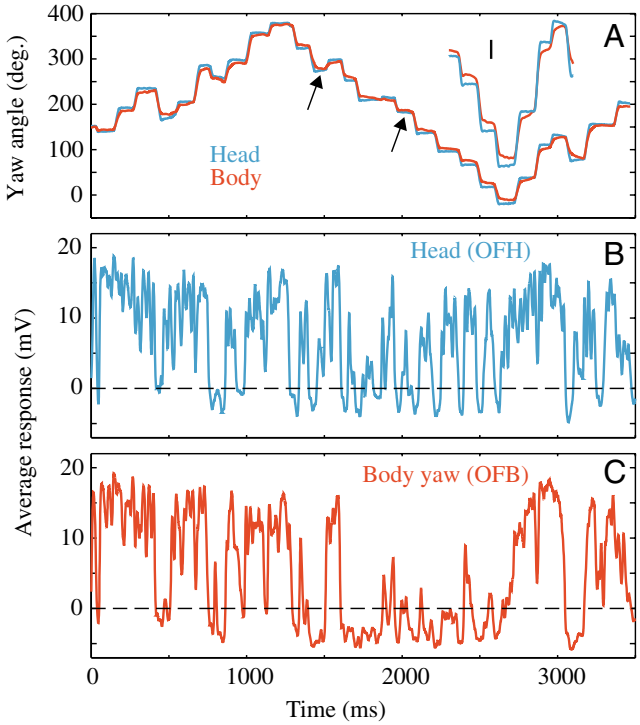

Fig. 1. (A) Time course of head (blue) and body (red) yaw angle exemplifying the saccadic flight style of blowflies. Positive slopes denote leftward turns, i.e. turns leading to optic flow into the preferred direction of the right horizontal system equatorial neuron (HSE). The free-flight data were recorded in an arena with dimensions of about 40×40×40 cm^3, with images of herbage covering the walls. Arrows are pointing towards instances where slow, intersaccadic angular head movements were against (left arrow) or with (right arrow) the direction of previous saccadic turns. (Inset) Sections of yaw traces, vertically enlarged (scale bar, 20°). (B,C) Average membrane potential of an HSE-cell in the right brain hemisphere in response to the optic flow corresponding to (B) head or (C) body movements, the yaw component of which is shown in A (N=9 responses each). Broken lines denote resting potential; responses are shifted backwards in time to account for response latencies and low-pass filtered with a Gaussian, standard deviation of 3 ms. Upward and downward deflections of the yaw angle in A correspond to optic flow in the preferred and null direction of the analysed neurons, respectively.

same direction as that of the previous saccade (note the slope of the red line in intersaccadic intervals; Fig. 1A inset). By contrast, the head usually reaches an orientation close to its final orientation immediately at the end of the rapid saccadic movement (rather stable yaw angle in intersaccadic intervals; Fig. 1A inset, blue line) (see also van Hateren and Schilstra, 1999). Quite frequently, the head may either over- or undershoot its mark by a few degrees (see arrows in Fig. 1A) and then performs a slight angular drift during the intersaccadic interval. On average, for a large number of saccades observed in a large number of flies, however, there is no significant over- or undershooting (Schilstra and van Hateren, 1998; van Hateren and Schilstra, 1999). In any case, head yaw orientation is considerably more constant between saccades than body yaw orientation.

As has been shown previously, changes in the yaw angle during a saccade are paralleled by body roll movements that, depending on the saccade amplitude, may reach an angle of up to 90° (Schilstra and van Hateren, 1999). Since body roll is largely counteracted by compensatory roll movements of the head (Hengstenberg, 1988; van Hateren and Schilstra, 1999), it has only limited impact on the optic flow experienced by the eyes. Furthermore, neurons primarily sensitive to horizontal motion will respond strongly to yaw rotations but considerably less to roll rotation. Since we want to focus in this article on the functional consequences of yaw rotations of body and head for the horizontally sensitive neurons, we will not further consider roll movements, but instead assume perfect roll compensation. Similarly, the pitch angle will be assumed to be constant as well (see Materials and methods for details).

Neuronal responses to optic flow based on head and body movements

How different are the responses of motion-sensitive neurons to the optic flow resulting from either the head movements (abbreviated as OFH below) or the optic flow resulting from the yaw movements of the body (OFB)? Average responses of an HSE-cell are shown in Fig. 1B,C. The responses to both OFH and OFB are characterised by pronounced fluctuations in the graded membrane potential (Hausen, 1982a; Haag et al., 1999). Since in either variant the behaviourally generated optic flow has a complex temporal structure, the time course of the corresponding neuronal responses is complex as well. It is therefore hard, at first sight, to infer any immediate conclusions about what stimulus features the cell may encode under the two conditions. The responses to the two optic flow variants are similar in some respects, but there are also large differences. Neither head nor body saccades that lead to pronounced horizontal front-to-back motion (upward deflections of the yaw angle in Fig. 1A) evoke obvious depolarisations, although such depolarisations might be expected from the neuron's physiological properties inferred from responses to simple experimenter-defined stimuli (Haag and Borst, 1997; Hausen, 1982a; Hausen, 1982b; Horstmann et al., 2000). Only during saccades that go along with (inhibitory) back-to-front motion (downward deflections of the yaw angle in Fig. 1A) are clear hyperpolarising peaks induced, at least in the responses to OFH (see also Kern et al., 2005). Note that HSE-cells are depolarised between saccades for extended times during stimulation with either OFH or OFB, even though the overall optic flow between saccades is only small compared with the optic flow generated during saccades. The responses to OFH and OFB are clearly different between ~1700 and 2600 ms in the traces shown in Fig. 1, where the intersaccadic rotation of the body in the null-direction of the HSE cell (Fig. 1A) hyperpolarised the response to OFB. The corresponding response to OFH, on the other hand, is much more depolarised between the saccades. Since these depolarisations in the responses to OFH have been shown previously to be the consequence of translational optic flow (Kern et al., 2005) and thus of the spatial structure of the environment, the saccadic gaze strategy has been concluded to be a specialisation that enables the extraction of translatory optic flow amidst rotatory optic flow (Kern et al., 2005; van Hateren et al., 2005). Therefore, we concentrate in the following on the question of what behaviourally generated information is encoded by motion-sensitive neurons between saccades.

Just from scrutinising the time courses of the intersaccadic responses to OFH and OFB, it is hard to tell whether their differences matter from a functional point of view. Therefore, intersaccadic-response segments were analysed quantitatively after masking the saccadic segments of stimulus and response (see Materials and methods) (Kern et al., 2005; van Hateren et al., 2005). We determined the optimal linear filters for estimating (reconstructing) self-motion parameters from the responses and quantified the similarity between estimated and original self-motion parameters by the coherence, which varies between zero (i.e. at frequencies where both signals are not correlated) and one (i.e. perfect reconstruction). The coherence function is thus a measure of the ability of the HSE-cell to provide the animal with information on its self-motion parameters from the intersaccadic optic flow. The coherence functions for OFH and OFB are quite different. For the optic flow based on head movements, i.e. the actual optic flow that was seen by a fly while flying around in the cage, the coherence of the intersaccadic yaw velocity and the neuronal response was substantial only between approximately 20 and 60 Hz (Fig. 2A, broken line). There was considerable coherence between sideward velocity and the neuronal responses at low frequencies (Fig. 2A, solid line). This result, based on four HSE-cells, is in accordance with previous findings on HSE-cells and shows that, in principle, information on sideward translation and yaw rotation can be separated on the basis of the divergent frequency dependence (Kern et al., 2005). If the optic flow is reconstructed from the body yaw (OFB), rather than from the head movements, the corresponding coherences are not only quantitatively different, but also qualitatively. Now, the coherence for yaw rotations is largest in the low-frequency range (Fig. 2B, broken line), and it is no longer possible to separate sideward translation and yaw rotations on the basis of different frequency allocations. The same conclusions can be drawn for three other types of blowfly

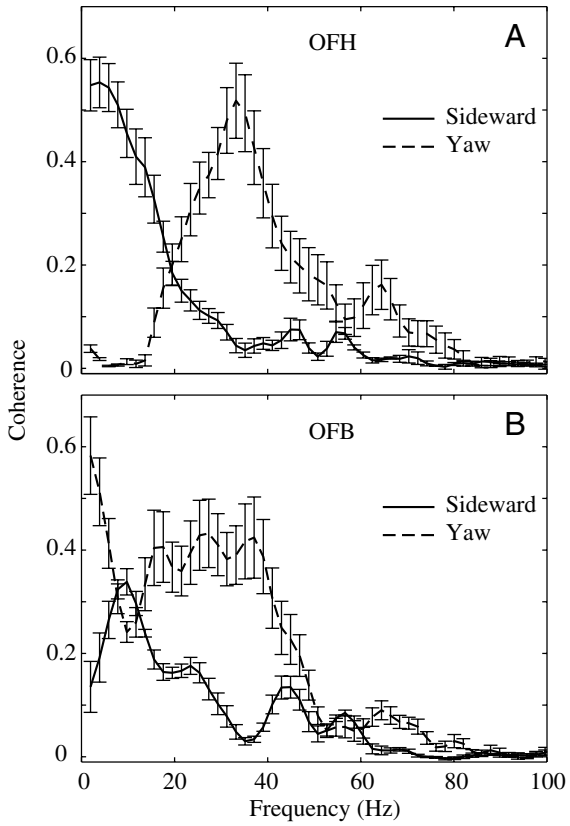

Fig. 2. Average coherence (± s.e.m.) of sideward angular velocity (solid line) and yaw velocity (broken line) with the subtracted responses of right and left horizontal system equatorial neuron (HSE) (*N*=4), intersaccadic parts of stimulus and response only. Responses to optic flow based on head orientation (A) or body yaw (B). Sideward is perpendicular to the head's plane of symmetry. Behavioural data used for reconstruction of visual stimuli are based on two flights originating from different flies.

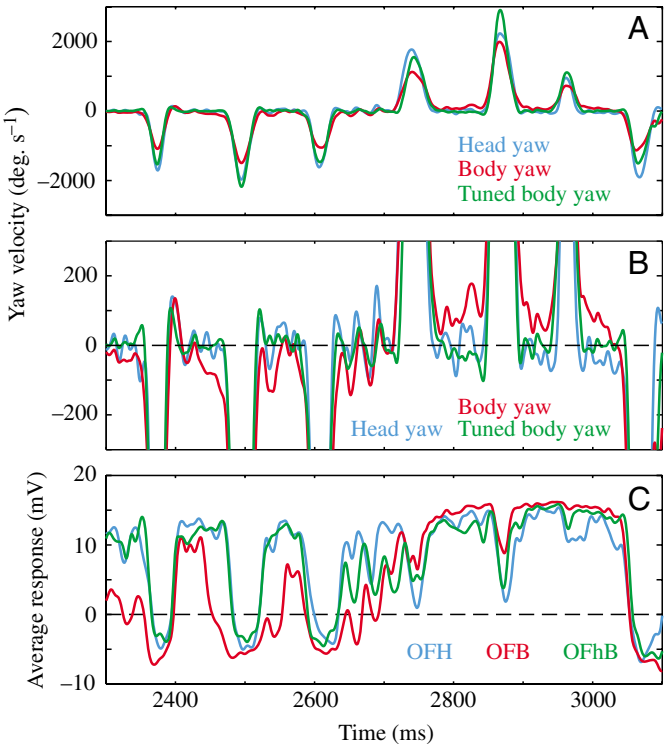

Fig. 3. (A) Yaw velocity of head (blue), body (red) and headified (yaw-tuned) body (green). This flight segment corresponds to the segment for which yaw angles are shown enlarged in Fig. 1A inset. (B) Same as A, vertically zoomed. Yaw velocities in A and B were obtained from the differential rotation matrix of the body or head (van Hateren and Schilstra, 1999) and were low-pass filtered with a Gaussian (standard deviation 3 ms) to reduce measurement noise. (C) Average response of an horizontal system south neuron (HSS) cell to optic flow corresponding to yaw velocities shown in A. Broken line at 0 mV corresponds to the resting potential; responses are shifted backwards by 22.5 ms to account for response latencies and low-pass filtered with a Gaussian, standard deviation 3 ms.

motion-sensitive cells (HSN, HSS and DCH). It was concluded in our previous study that it is a major function of HS-cells to extract, between saccades, information about translational movement of the fly and thus, indirectly, on the spatial layout of the environment (Kern et al., 2005). Our results from the present study reveal now that this conclusion would not have been drawn if only data on body movements had been available. This finding clearly shows that although head and body, at first sight, move relatively synchronously, the subtle differences between head and body movements are functionally highly relevant.

What are the reasons for the pronounced differences between the responses to optic flow reconstructed from head and body movements, respectively? To answer this question, we scrutinised the yaw movements of head and body in more detail. Several differences in the yaw velocity profiles during saccades become apparent: peak angular velocities reached during body saccades are smaller than during head saccades, and the body saccades tend to start slightly earlier and to terminate slightly later than head saccades (Fig. 3A, red and blue lines) (see also van Hateren and Schilstra, 1999). During the intersaccadic intervals, the yaw velocity of the head fluctuates around zero, with no or only small maintained rotations towards either side. Hence, the gaze is quite precisely stabilised apart from small-amplitude high-frequency fluctuations. By contrast, the body yaw velocity drifts much more between saccades and, in many cases, does not fluctuate around zero but stays for most of the intersaccadic interval at either a positive or negative level. As a consequence, if the gaze were estimated from the orientation of the blowfly's body long axis, considerable deviations from straight gaze would be inferred, whereas such deviations are not present in the head (Fig. 3B, red and blue lines). These differences in the time course of the head and body yaw rotations can be substantiated for the entire set of behavioural sequences used in the present study. The probability density function of body yaw velocity, calculated for the intersaccadic intervals, is broader than that of the head velocity (Fig. 4A, red and blue lines). Most importantly, in the low-frequency range, the power spectral

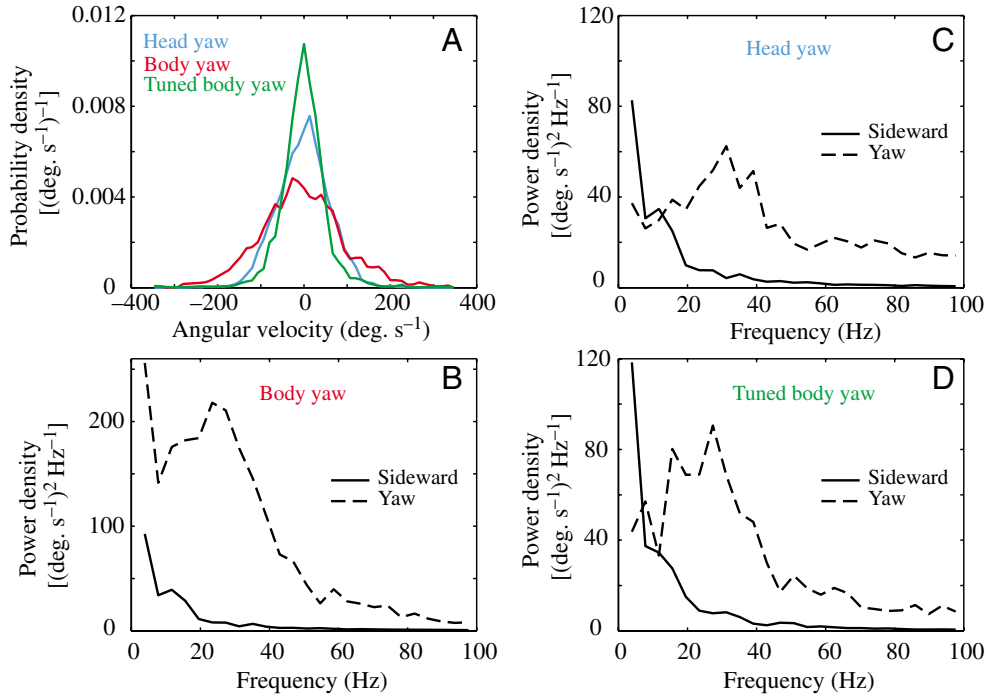

Fig. 4. (A) Probability density function at the intersaccadic intervals of head yaw (blue), body yaw (red) and tuned body yaw (green) velocity. (B–D) Power spectra of sideward (solid) and yaw (broken) angular velocity for body yaw velocity (B), head yaw velocity (C) and tuned body yaw velocity (D); power spectral densities were calculated for the intersaccadic intervals using an algorithm by (Scargle, 1989; see also Kern et al., 2005b). Sideward is perpendicular to the head's plane of symmetry. Since the optic flow resulting from translational movements depends on the distance to environmental objects, sideward velocities of the fly were converted to angular velocities by multiplying by the nearness (equal to the inverse of the distance) (Koenderink, 1986) averaged over the trajectories and over the receptive fields of the neuron (average nearness: 7.14 m^{-1}, corresponding to a typical distance to the arena wall of 0.14 m). Data based on two flights originating from different flies. Note that the *y*-axis in B is scaled differently from those in C and D.

density of body yaw velocity fluctuations, evaluated for the intersaccadic intervals (Kern et al., 2005), is much larger than the power of sideward velocity (Fig. 4B). As a consequence, the optic flow evoked by body yaw rotations dominates, for all frequencies, the optic flow that results from sideward motion. The corresponding power spectral densities for the actual head movements (Fig. 4C) show that the low-frequency body yaw rotations are largely compensated by head movements. The intersaccadic optic flow generated on the eyes is therefore dominated, in the low-frequency range, by sideward movements of the animal and not by yaw rotations. In principle, these features allow higher levels of the visual motion pathway to extract translational information from the responses of motion-sensitive neurons.

Simulation of yaw rotations of the head by temporal filtering of yaw movements of the body

The results described so far stress the significance of yaw-reducing head movements for stabilizing gaze during the intersaccadic intervals, which allow the visual system to access the translational optic flow. However, in studies on the significance of optic flow for orientation behaviour of insects, the head yaw may not be available. If camera systems are used to record the flights, usually only the yaw orientation of the body long axis can be resolved with sufficient accuracy, and not the head yaw. Since the behavioural data obtained with the magnetic coil system allow us to resolve both head and body orientation in great detail (Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999), we developed a simple algorithm to transform the time-dependent yaw rotations of the body into a yaw-tuned ('headified') signal, which comes reasonably close to the yaw rotations of the head. To test the algorithm, we determined the optic flow resulting from the headified body movements (OFhB) and compared the corresponding neuronal responses with those induced by the optic flow reconstructed from the real head movements. It should be noted that this filtering procedure is not intended to represent a model of yaw movements of the head. Rather, it is just a pragmatic approach to 'headify' the body movements, so that more realistic estimates of the optic flow are possible on the basis of methods only providing the orientation of the body long axis, but not the head.

The different steps to headify the time-dependent body orientation are summarised in Fig. 5. The yaw of the body is combined with an assumed zero roll and a fixed pitch. These so-called Fick-angles yield the rotation matrix (Haslwanter, 1995) as a function of time. The change in orientation per time step is then given by the differential rotation matrix, from

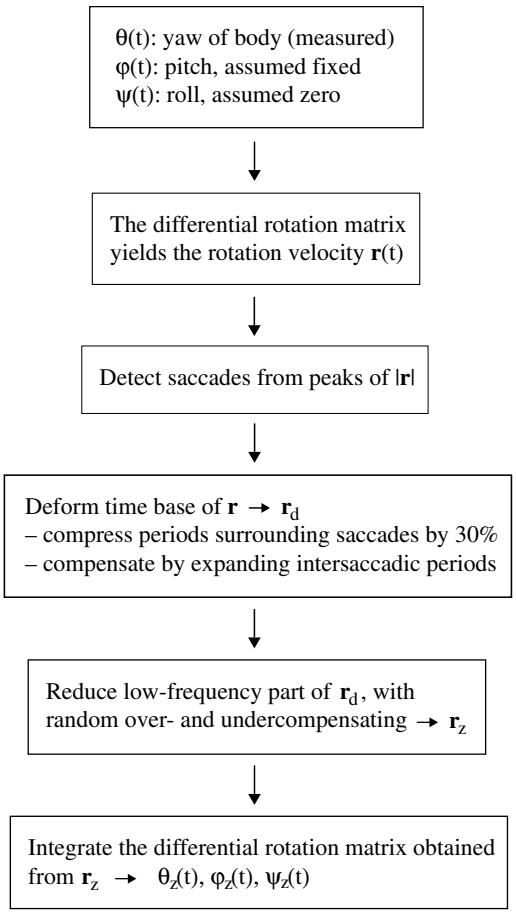

Fig. 5. Algorithm to obtain a headified body orientation from the measured body yaw. See the text for an explanation.

which the rotation velocity can be obtained (Haslwanter, 1995). Because the head saccades are on average approximately 30% faster and shorter than the body saccades (e.g. Fig. 3A), the latter are made faster and shorter by compressing the saccadic periods by 30% and expanding the intersaccadic periods as required to keep the times of occurrence of the saccades unchanged. The power of the low-frequency components of the intersaccadic yaw velocity of the head is much lower than that of the body (Fig. 4B,C). Nonetheless, just reducing this low-frequency band in the intersaccadic yaw velocity of the body by the headifying algorithm did not provide satisfactory results, i.e. did not allow recovering the sideward translational velocity from the overall optic flow. The reason is that the body nearly always shows an angular drift into the same direction as the previous saccade, and only reducing this drift would still keep this saccade-drift correlation intact. Because the saccade-drift relationship also produces a correlation between intersaccadic yaw velocity and sideward velocity, these parameters are not fully separable in the frequency domain, as shown by the coherences of control experiments with this stimulus. A correlation between yaw drift and sideward velocity is absent in the head, which shows angular drifts with equal probability into either the same or the opposite direction of the previous saccade. We therefore solved this problem by assuming per saccade a random over- or undercompensation of the head, whilst at the same time reducing the power spectral density of the body yaw velocity in an appropriate low-frequency band. Finally, the rotation velocity thus obtained yields a modified differential rotation matrix. By integrating this matrix under the condition that the yaw at the midpoint of each intersaccadic period is identical to the original body yaw, the Fick-angles of the headified body are obtained.

The results of the headifying algorithm for yaw velocity are shown in Figs 3, 4. The peak velocity during saccades is increased relative to the body saccades and thus, in most situations, comes closer to the yaw velocity of head movements (Fig. 3A, green and blue lines). Most importantly, the pronounced slow yaw rotations of the body between saccades are largely eliminated. The headified body orientation is consequently much steadier than body orientation and resembles the real head orientation more closely, because it mainly fluctuates around zero yaw velocity (Fig. 3B, green line). Accordingly, the angular velocities of the headified body rotations are confined to smaller values than the original body rotations (Fig. 4A, green line). In particular, the reduction is most pronounced in the low-frequency range. As a consequence, the power of sideward velocity is now much larger for these frequencies than the power of body yaw velocity fluctuations (Fig. 4D). In conclusion, although the angular velocities of the headified body rotations still differ in many details from the real head rotations, they match qualitatively in the most relevant features. Both the real head and headified body rotations show similar frequency optima of the power density of sideward translation and yaw rotation (compare Fig. 4C,D).

Are these similarities between the headified and real head rotations reflected in similar neuronal responses? This question was posed for a number of blowfly tangential cells sensitive to horizontal motion. Fig. 3C shows an example of the response of an HSS cell to OFH (blue line), OFB (red line) and OFhB (green line). Although there are still differences, it is clear that the OFhB response comes much closer to the OFH response than the response to the OFB does. The relationship between neuronal responses and both yaw and sideward velocity was, as above, quantified by calculating coherence functions. These are shown in Fig. 6 for three cell types (HSE, HSS and DCH) and the three stimulus conditions (OFH, OFB and OFhB). Despite quantitative differences, the results are qualitatively similar for all analysed cell types (including HSN; not shown). In the responses to OFH, the coherence of all cell types for sideward translation is much higher in the low-frequency range than for yaw rotation. For velocity fluctuations at frequencies above 15–20 Hz, this relationship is reversed and the coherence for yaw rotations is larger than for sideward translation, allowing for a separation of both self-motion parameters on the basis of the frequency content of the neuronal responses (Fig. 6A,D,G). This functionally important consequence of head movements is not retained in the

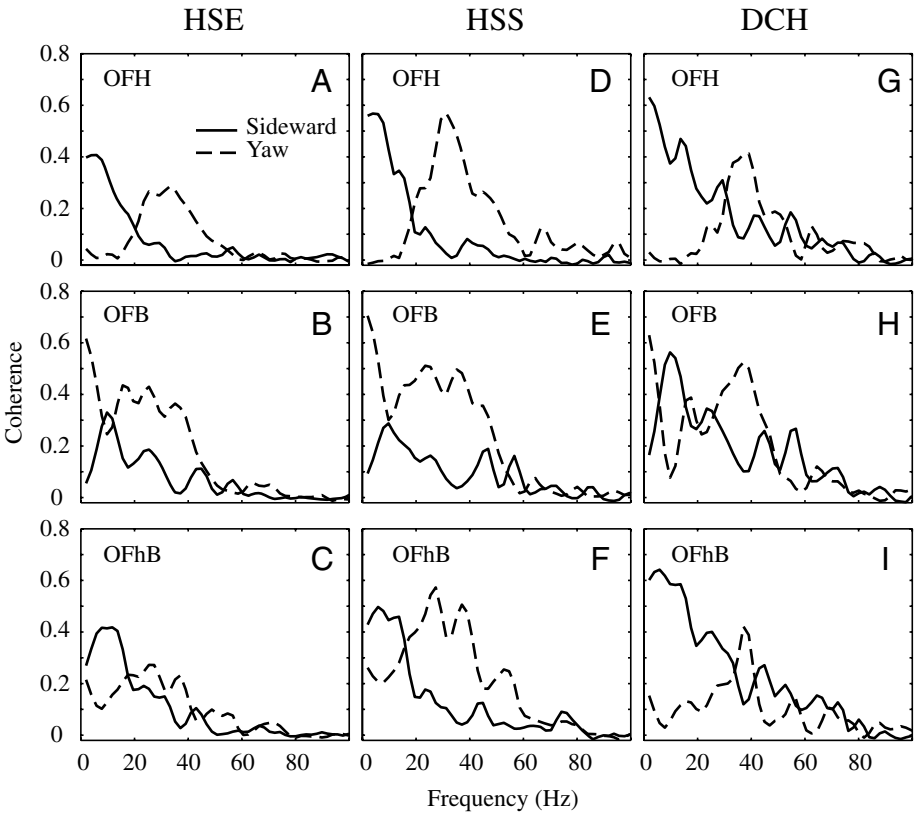

Fig. 6. Coherence of sideward angular velocity (solid line) and yaw velocity (broken line) with the subtracted responses of right and left horizontal system equatorial neuron (HSE) (A–C), horizontal system south neuron (HSS) (D–F) and dorsal centrifugal horizontal neuron (DCH) cell (G–I), intersaccadic parts of stimulus and response only. Responses to optic flow based on head (A,D,G), body (B,E,H) or yaw tuned body (C,F,I) movements. Single cell results, based on 3–8 stimulus repetitions of two different flights. Sideward is perpendicular to the head's plane of symmetry.

responses to OFB but reappears in the responses to OFhB, although it is not as pronounced as in OFH. Hence, the headifying procedure presented here is successful in utilizing body movements to recover, at least to a large extent, the functionally important features of the optic flow as generated by eye movements.

Discussion

Optic flow has been known for a long time to be an important source of information about the spatial layout of the environment. Distance information, however, is only contained in the translational optic flow component (Koenderink, 1986). If extracted by the nervous system, it can be used by an animal to guide its orientation behaviour. For rapidly flying animals, optic flow information is, most likely, the only cue that can be used to recover information about spatial relationships. However, this requires the translational optic flow component to be separated from the rotational one. Although this is possible, at least in principle, in situations where translational and rotational movements occur simultaneously (Koenderink and van Doorn, 1987), animals may employ strategies that are simpler from the point of view of visual computation by using their own behaviour to separate rotational and translational optic flow. For instance, blowflies squeeze most of the rotational optic flow into saccades, while in the intersaccadic time interval the gaze is kept basically stable (Schilstra and van Hateren, 1999; van Hateren and Schilstra, 1999). As a consequence, the visual system experiences mainly translational optic flow in the intersaccadic intervals, at least at low frequencies and in flight circumstances with sufficiently close objects and sufficiently high flight velocities (see van Hateren et al., 2005).

Although this idea appears to be rather simple, its implementation in reality may be quite demanding. This is because without sensory feedback control most biological and technical systems do not move straight. Inherent asymmetries, such as not exactly matched forces of the two wings in the case of blowflies, are reflected in the animal's behaviour. Hence, feedback control is required to ensure stable gaze between saccades by compensating the residual rotations. Given the inevitable delays and time constants of any sensory-motor system, it is a hard task for a feedback control system to compensate rotational movements within the short time interval between saccades, which may be as small as 50 ms.

Nonetheless, blowflies achieve this goal sufficiently well, since between saccades head yaw velocities are smaller than the sideward velocities up to frequencies of 15–20 Hz, allowing visual interneurons sensitive to horizontal motion to extract the relevant translational information (see also Kern et al., 2005). This is true right from the beginning of the intersaccadic interval (Figs 1A, 3B), suggesting either a feedforward or an extremely fast feedback control system. In blowflies, both body and head movements are involved in gaze stabilisation between saccades. However, gaze stabilisation is not fully accomplished by body movements on their own, but for a significant part by very precise head movements. Accordingly, the precise head–body coordination is essential

for the visual system to separate the translational from the rotational optic flow and for using this information for spatial orientation. If the head were tightly coupled to the body, the resulting optic flow would not contain the behaviourally relevant information. This finding raises two questions that will be addressed in the following. (1) How are the compensatory yaw rotations of head and body controlled? (2) Can the time course of head rotations be inferred from body rotations?

There are, in principle, two different sensory modalities that may provide the relevant input to the feedback control system stabilising gaze in the intersaccadic interval: the visual system and the mechanosensory haltere system. The halteres are the evolutionarily modified hind wings of flies. Both systems have been shown to mediate compensatory head rotations. Although yaw rotations of the head can be evoked visually (Land, 1973), the visual feedback loop is likely to be too slow to play the major role in compensating yaw rotations within the relatively short intersaccadic interval. The latencies at the level of motion-sensitive cells that were analysed here are already in the range of 20 ms (Warzecha and Egelhaaf, 2000) and are likely to be even larger at the motor output. Nonetheless, we cannot exclude that the optic flow experienced during the intersaccadic intervals and generating large responses in motion-sensitive visual interneurons (e.g. Fig. 1C) plays an assisting role in inter-saccadic head stabilisation.

The mechanosensory haltere system acts similarly to a gyroscopic sensor (Pringle, 1948). With this system, blowflies can discriminate between angular velocities about all axes of their body and use this information to make appropriate compensatory adjustments in head orientation (Nalbach and Hengstenberg, 1994; Nalbach, 1993). The haltere system is sensitive to higher velocities than the visual system (Hengstenberg, 1988; Sherman and Dickinson, 2003). With behavioural latencies between 5 and 10 ms, a feedback loop fed by haltere input is likely to be sufficiently fast to compensate the residual body yaw rotations between saccades up to frequencies of 15 Hz (Nalbach and Hengstenberg, 1994; Nalbach, 1993).

Why are low-frequency yaw rotations up to 10–15 Hz of the body between saccades much less precisely compensated than those of the head, even though haltere feedback affects the wing stroke parameters relevant for steering the body as rapidly as head movements (Nalbach and Hengstenberg, 1994; Nalbach, 1993)? The likely reason is the much greater mass and, accordingly, greater inertia of the body compared with that of the head. Making the body as stable as the head would require considerable forces and therefore considerably more energy than is required when part of the stabilization is performed by the head. Nonetheless, flies possess a robust, haltere-mediated equilibrium reflex in which angular rotations of the body elicit compensatory changes in both the amplitude and stroke frequency of the wings and function primarily to stabilise pitch and yaw of the body within the horizontal plane (Dickinson, 1999).

Our finding that behaviourally relevant translational information can only be recovered from the corresponding neuronal responses if head movements are taken into account for determining the optic flow patterns may be relevant from a methodological point of view. As a consequence of methodological limitations of film and video technologies, almost all free-flight studies on visually guided orientation behaviour of insects infer optic flow information from the time course of the location of the animal in space (Land and Collett, 1974; Zeil, 1986) or of the yaw angle of the body long axis (Collett, 1980a; Collett, 1980b; Collett and King, 1975; Wagner, 1986a; Wagner, 1986b; Wagner, 1986c; Zeil, 1993a; Zeil, 1993b; Zeil et al., 1997; Boeddeker et al., 2005; Olberg et al., 2000; Lehrer, 1991; Lehrer and Srinivasan, 1992). Depending on the question studied, such inferences can be problematic given the qualitative differences found for the optic flow and its neuronal representation when using either head or body movements. Even current digital video techniques are in most situations not sufficient to resolve head orientation when flight behaviour is analysed in a reasonably large area. The algorithm developed here to transform the time-dependent body orientation in free flight into an estimate of head orientation may therefore be useful to alleviate this problem and to provide an acceptable estimate of the retinal optic flow pattern. Under the conditions of free flight tested here, the headifying algorithm could recover those features of optic flow that were concluded, on the basis of the real head movements, to be behaviourally relevant. Nonetheless, it should be noted that the fly performs better than the proposed algorithm in separating translational from rotational flow between saccades (Fig. 6). We also note that the fly has a lot more sources of sensory and internal information relevant for gaze stabilisation than we could use here for our algorithm.

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